

SEQUENCE LISTING

<110> Rosen, Craig A.
Haseltine, William A.

<120> Albumin Fusion Proteins

<130> PF548

<140> Unassigned

<141> 2001-04-12

<150> 60/229,358

<151> 2000-04-12

<150> 60/256,931

<151> 2000-12-21

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<151> 2000-04-25

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<223> first 5 amino acids of mature human serum albumin

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1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser
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<213> Artificial Sequence

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 gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag 96
 Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
 20 25 30
 cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa 144
 Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
 35 40 45
 ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
 Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
 50 55 60
 tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt 240
 Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65 70 75 80
 cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
 Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
 85 90 95
 gag aga aat gaa tgc ttc ttg caa cac aaa gat gac aac cca aac ctc 336
 Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
 100 105 110
 ccc cga ttg gtg aga cca gag gtt gat gtg atg tgc act gct ttt cat 384
 Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
 115 120 125

gac aat gaa gag aca ttt ttg aaa aaa tac tta tat gaa att gcc aga Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg 130 135 140	432
aga cat cct tac ttt tat gcc ccg gaa ctc ctt ttc ttt gct aaa agg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg 145 150 155 160	480
tat aaa gct gct ttt aca gaa tgt tgc caa gct gct gat aaa gct gcc Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala 165 170 175	528
tgc ctg ttg cca aag ctc gat gaa ctt cgg gat gaa ggg aag gct tcg Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser 180 185 190	576
tct gcc aaa cag aga ctc aaa tgt gcc agt ctc caa aaa ttt gga gaa Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu 195 200 205	624
aga gct ttc aaa gca tgg gca gtg gct cgc ctg agc cag aga ttt ccc Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro 210 215 220	672
aaa gct gag ttt gca gaa gtt tcc aag tta gtg aca gat ctt acc aaa Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys 225 230 235 240	720
gtc cac acg gaa tgc tgc cat gga gat ctg ctt gaa tgt gct gat gac Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp 245 250 255	768
agg gcg gac ctt gcc aag tat atc tgt gaa aat cag gat tcg atc tcc Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser 260 265 270	816
agt aaa ctg aag gaa tgc tgt gaa aaa cct ctg ttg gaa aaa tcc cac Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His 275 280 285	864
tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser 290 295 300	912
tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala 305 310 315 320	960
gag gca aag gat gtc ttc ctg gcc atg ttt ttg tat gaa tat gca aga Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg 325 330 335	1008
agg cat cct gat tac tct gtc gtg ctg ctg ctg aga ctt gcc aag aca Arg His Pro Asp Tyr Ser Val Val Leu Leu Arg Leu Ala Lys Thr 340 345 350	1056
tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu 355 360 365	1104

tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct	1152
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro	
370 375 380	
cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag	1200
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu	
385 390 395 400	
tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc	1248
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro	
405 410 415	
caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa	1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys	
420 425 430	
gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt	1344
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys	
435 440 445	
gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat	1392
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His	
450 455 460	
gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc	1440
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser	
465 470 475 480	
ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca	1488
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr	
485 490 495	
tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat	1536
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp	
500 505 510	
ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca	1584
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala	
515 520 525	
ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg	1632
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu	
530 535 540	
aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag	1680
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys	
545 550 555 560	
gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt	1728
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val	
565 570 575	
gct gca agt caa gct gcc tta gcc tta taacatctac atttaaaagc atctcag	1782
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<213> Homo Sapiens

<400> 18

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20 25 30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35 40 45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50 55 60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65 70 75 80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85 90 95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
115 120 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
130 135 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145 150 155 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
165 170 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
180 185 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
195 200 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
210 215 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225 230 235 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
245 250 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
260 265 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
275 280 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
290 295 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
 305 310 315 320
 Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
 325 330 335
 Arg His Pro Asp Tyr Ser Val Val Leu Leu Arg Leu Ala Lys Thr
 340 345 350
 Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
 355 360 365
 Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
 370 375 380
 Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
 385 390 395 400
 Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
 405 410 415
 Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
 420 425 430
 Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
 435 440 445
 Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
 450 455 460
 Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
 465 470 475 480
 Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
 485 490 495
 Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
 500 505 510
 Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
 515 520 525
 Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
 530 535 540
 Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
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 site in pPPC0006

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<210> 20
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<400> 21
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<210> 22
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 site in pPPC0006

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<223> Synthetic oligonucleotide used to alter restriction sites in pPPC0007

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<210> 24

<211> 60

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<223> forward primer useful for generation of albumin fusion protein in which the albumin moiety is N-terminal of the Therapeutic Protein

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<223> reverse primer useful for generation of albumin
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the Therapeutic Protein

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<210> 29
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<220>
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Tyr Ser Arg Ser Leu Asp Lys Arg
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 <222> (5)..(10)
 <223> BamHI restriction site

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 <222> (11)..(16)
 <223> Hind III restriction site

<220>
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 <222> (17)..(27)
 <223> Kozak sequence

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<220>
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<222> (25)..(97)
<223> cds natural signal sequence of human serum albumin

<220>
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<222> (75)..(81)
<223> XhoI restriction site

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<222> (98)..(114)
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<400> 30
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tttagctcgg cttactcgag ggggtgtgtt cgtcgagatg cacacaagag tgaag      114

<210> 31
<211> 43
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<220>
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<220>
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<222> (12)..(17)
<223> EcoRI restriction site

<220>
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<222> (15)..(17)
<223> reverse complement of stop codon

<220>
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<222> (18)..(25)
<223> AscI restriction site

<220>
<221> misc_feature
<222> (18)..(43)
<223> reverse complement of DNA sequence encoding last 9 amino acids

<400> 31
gcagcgggtac cgaattcggc ggcgccttata agcctaaggc agc      43

<210> 32

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<211> 46
<212> DNA
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<220>
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<223> forward primer useful for inserting Therapeutic
protein into pC4:HSA vector

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<400> 32
ccgccgctcg aggggtgtgt ttcgtcgann nnnnnnnnnn nnnnnn

46

<210> 33
<211> 55
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<222> (55)

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<210> 34

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> signal

<223> Stanniocalcin signal peptide

<400> 34

Met Leu Gln Asn Ser Ala Val Leu Leu Leu Leu Val Ile Ser Ala Ser
1 5 10 15

Ala

<210> 35

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<221> signal

<223> Synthetic signal peptide

<400> 35

Met Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Ala Leu
1 5 10 15

Trp Ala Pro Ala Arg Gly
20

<210> 36

<211> 23

<212> DNA

<213> Artificial Sequence

<220>
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 <223>Degenerate VH forward primer useful for
 amplifying human VH domains

 <400> 36
 caggtgcagc tgggtgcagtc tgg 23

 <210> 37
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
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 <400> 37
 caggtcaact taaggagtc tgg 23

 <210> 38
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
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 <400> 38
 gaggtgcagc tgggtggagtc tgg 23

 <210> 39
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 <400> 39
 caggtgcagc tgcaggagtc ggg 23

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 <212> DNA
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<400> 40
gaggtgcagc tgttcagtc tgc 23

<210> 41
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<400> 41
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<210> 42
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<212> DNA
<213> Artificial Sequence

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amplifying human VH domains

<400> 42
tgaggagacg gtgaccaggg tgcc 24

<210> 43
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
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amplifying human VH domains

<400> 43
tgaagagacg gtgaccattg tccc 24

<210> 44
<211> 24
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<213> Artificial Sequence

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<400> 44
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<210> 45
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 <400> 45
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 <210> 46
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 <220>
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 <400> 46
 gacatccaga tgaccagtc tcc 23

 <210> 47
 <211> 23
 <212> DNA
 <213> Artificial Sequence

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 <210> 52
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 <210> 53
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
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 <400> 53
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 <210> 54
 <211> 23
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 <213> Artificial Sequence

 <220>
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<223>Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 54
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<210> 55
<211> 23
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<400> 55
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<210> 56
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<220>
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<400> 56
tcttctgagc tgactcagga ccc 23

<210> 57
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<212> DNA
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<400> 57
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<210> 58
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<220>
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<400> 58
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<210> 59
<211> 23

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<212> DNA
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23

<210> 60
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<210> 61
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24

<210> 62
<211> 24
<212> DNA
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<400> 62
acgtttgata tccactttgg tccc
24

<210> 63
<211> 24
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<220>
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amplifying human VL domains

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<400> 63
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 <210> 64
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 <400> 64
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 <210> 65
 <211> 23
 <212> DNA
 <213> Artificial Sequence

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 <400> 65
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 <210> 66
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
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 <400> 66
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 <210> 67
 <211> 23
 <212> DNA
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 <220>
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 <400> 67
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 <210> 68
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 <400> 68
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 <210> 69
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 <400> 69
 cacgttatac tgactcaacc gcc 23

 <210> 70
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 <220>
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 <400> 70
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 <210> 71
 <211> 23
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 <213> Artificial Sequence

 <220>
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 <400> 71
 aattttatgc tgactcagcc cca 23

 <210> 72
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <221>turn
 <223>Linker peptide that may be used to join VH
 and VL domains in an scFv.

 <400> 72
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15